METHANOGEN COMMUNITIES IN PEATLANDS OF NORTH AMERICA

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by
SYDNEY ELIZABETH BEAR

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by
SYDNEY E. BEAR
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APPROVED BY:

Dr. Suzanna L. Bräuer
Chairperson, Thesis Committee

Dr. Nathan Basiliko
Member, Thesis Committee

Dr. Cortney Bouldin
Member, Thesis Committee

Dr. Zack Murrell
Chairperson, Department of Biology

Mike McKenzie, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

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Sydney E. Bear

B.S., Appalachian State University
M.S., Appalachian State University

Chairperson: Dr. Suzanna L. Bräuer

` Peatlands are unique wetlands that play a role in the storage and release of atmospheric carbon in the form of carbon dioxide and methane gas. Peatland environments are estimated to account for one third of Earth's terrestrial carbon. Peatland soil contains communities of archaea, bacteria, and fungi that interact with each other through nutrient cycling and competition. The methanogenic archaea in peatland communities create methane gas as a product of their metabolism. A community analysis of methanogen groups and their community make-up in varying peatland environments was conducted to provide insight into their interactions and how the changing environment will affect them. Extracted DNA from peat samples of 17 sites in eastern North America was analyzed through Illumina amplicon sequencing of the mcrA gene, as well as 16S rRNA, to observe changes in methanogenic community assemblages in varying environments. Methanogen community diversity was seen to increase with depth and for peatland classifications with higher nutrient concentrations. The community structure of the peatlands showed that the more acidic peatlands had prevalence of Methanomassiliicoccous, Methanomicrobiales, and Methanocellales, which are common in ombrotrophic bogs. The less acidic to pH neutral peatlands showed a prevalence of Methanobacteria and Methanosaeta, which are associated

with fens. Metal concentrations in the soils also proved to be a driver of community diversity, with Ni being the most prevalent. The methanogen groups Methanocellales and Methanosarcina were seen to only be prevalent in peatlands with low Ni concentration, while the more versatile group Methanomicrobiales can thrive in peatlands with high Ni concentrations. The effect of environmental factors on the community structure of the peatlands shows how the preferences of methanogenic groups can drive diversity in peatlands. These data may provide insight into the community make-up and interactions of methanogen communities in peatland environments and are important to consider in the face of climate change.

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Dedication

I dedicate this thesis to my parents Karen and Bill Bear who have given me the support and encouragement I need in to be successful in my academics.

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Foreword

Chapter 2 of this thesis will be submitted to *FEMS Microbiology Ecology*, an international peer-reviewed forum owned and published by *Oxford University Press;* it has been formatted according to the style guide for that journal.

Chapter 1: Introduction

Peatlands are the most prevalent wetland in the world, covering 2.4 to 4.1 million km^2 (Bradley 2001). The United States and Canada together hold an estimated 40-45% of the world's peatlands, totaling to 1.86 million km^2 of peatland area (Vitt 2016). The second largest peatland ecosystem on Earth is found in Canada, in the James Bay and Hudson Bay lowlands (Kremenetski et al. 2003). Peatlands in northern Canada were formed around 5,600-11,000 years ago as a result of retreating glaciers (Zoltai and Tarnocai 1975), however the southern Appalachian peatlands are estimated to be much older, estimated to have formed around 300 million years ago (Cecil et al. 1985). Although peatlands occupy only 3% of the land area in the world, they are considered to be carbon sinks as they hold an estimated 1/2 of the earth's soil carbon (Gorham 1991; Gorham and Jansens 1992; Lehner and Döll 2004; Schlesinger and Bernhardt 2013).

Peatlands are classified by water source, water chemistry, and vegetation (Meindl 2005). Wetlands classified as bogs have acidic water, thick *Sphagnum* moss, and receive water from rain (ombrotrophic). These peatlands will have little to no water coming from the ground or surface, therefore their nutrients come primarily from atmospheric deposition and internal recycling of nutrients (Mitsch and Gosselink 1986). Wetlands classified as fens have higher pH, nutrients, plant diversity, and also receive influx from groundwater (Bradley 2001). In contrast to bogs, fens receive nutrient input from ground water and therefore are considered to be mineotrophic (Mitsch and Gosselink 1986). Fens can be further classified as rich (eutrophic/mesotrophic) or poor (oligotrophic) depending on their nutrient levels, including calcium, manganese, and phosphorus (Tahvanainen 2004). As a wetland moves from bog to fen

classification, there is an increase in the peatland decomposition rates, becoming closer to net rates of primary production (and leading to less peat accumulation), likely due to the higher levels of microbial activity seen in peatlands with higher nutrients and soil pH (Thormann et al. 1999; Wieder 1985).

Peatlands are characterized by the accumulation of organic matter which is called peat. Peat forms due to the imbalance of the biodegradation and the net primary productivity in these wetland soils (Williams and Crawford 1983). Peatlands have low decomposition rates because of their low soil pH, low nutrient levels, lack of oxygen, and water saturation (Freeman et al. 1996). The slow decomposition allows for the build-up of carbon in peatlands, which when released can be in the form of methane or carbon dioxide. The anaerobic conditions of peatlands make environments that harbor microbial methane production. Methane is a greenhouse gas that is important to consider in carbon cycling and climate change as it has a global warming potential that is 23 times higher than that of carbon dioxide, meaning that small increases in atmospheric methane can have a large impact on climate change (Roulet et al. 2007). The concentration of methane also appears to be increasing faster (relative to each pool size) than that of carbon dioxide in the atmosphere recently (Schlesinger and Bernhardt 2013). Atmospheric methane is largely a result of anthropogenic activities such as landfill use and burning of fossil fuels, however wetlands are estimated to contribute 20-30% of total methane emissions (Bloom et al. 2010; Bousquet et al. 2006; Ringeval et al. 2010). Although much of the carbon in wetlands is not fully decomposed, it can be sensitive to changes that could results in rapid decomposition and atmospheric release. Methane production in peatlands can vary substantially across seasons and inter-annually, but also with changing climate and other stressors (Steele et al. 1987). This can be a result of the ability of peatlands microorganisms to adapt to

environmental fluxes and therefore change their microbial community dynamics in a wetland environment (Fuhrman 2009). These changes are important to consider as these environments that have potential to hold and release large amounts of carbon could be affected by climate change and anthropogenic stress.

There are a variety of microbes with physiological activities that are detected in peat. The methane production of a peatland is a result of the balance between methanogens and methane oxidizing microbes (Lai 2009). There are microbial interactions in peatlands that occur in both the aerobic and anaerobic zones of the soil. Methanogens thrive in the anerobic soils while methane oxidizing microbes thrive in the oxygen rich soils. These zones are usually differentiated by the depth of the water table, as the methanogenic zone is generally found just below it at a depth around 40cm (Cadillo-Quiroz et al. 2006; Cadillo-Quiroz 2008; Galand et al. 2002).

The activities of these microbial groups can be affected by abiotic factors in the environment such as water table position, temperature, and pH (Lupascu et al. 2012). These abiotic shifts can also lead to changes in the vegetation that is present in peatlands, such as *Sphagnum* moss (aka peat moss) or sedge plant coverage. Peatlands that are dominated by *Sphagnum* can quickly change their community structure as the vegetation shifts. This likely has an effect on the soil pH and nutrient availability, which can shift methanogen communities between being dominated by acetoclastic or hydrogenotrophic methanogens (Kotsyurbenko et al. 2007; Rooney-Varga et al. 2007). Microorganisms can respond quickly to environmental changes, causing shifts of microbial diversity which can have an effect on the carbon cycling and emissions (Fuhrman 2009).

Methanogens are anaerobic Archaea that span 8 known orders with cultured representatives. This includes methanogens from phyla *Euryarchaetoa, Halobacteria*, and *Thermoplasmata*, with *Chrenarchaeota* containing candidate methanogens (Vanwonterghem, et al. 2016). Of these 8 orders, 5 are known to be common in peatlands: *Methosarcinales, Methanocellales , Methanbacteriales, Methanomicrobiales*, and *Methanomassiliicoccales.* Methanogens can be seen distributed globally in many different environments, including environments of extreme pH or temperature (Bräuer et al. 2006; Kamagata and Mikami 1991). They can be found in a variety of habitats, such as peatlands, rice paddies, landfills, sewage, hot springs, sediments, and digestive tracts (Chaban et al. 2006). Methanogens are unique as they are the only known organisms that contain the entire methanogenesis pathway (Gribaldo and Brochier-Armanet 2006). Methanogens all share the same final steps of their metabolism, which suggests that they all descend from a single common ancestor (Deppenmeier et al. 1996). The methanogenic functional gene that codes for the α subunit of the methyl coenzyme-M reductase (*mcrA*), which is used in the terminal steps of methanogenesis, allows for the detection and taxonomic characterization of methanogens in microbial community studies (Luton et al. 2002).

The methanogenesis pathway is the last step of anaerobic decomposition. The process of methanogenesis occurs through a variety of metabolic pathways that relies on a limited number of substrates derived that are byproducts of bacterial fermentation reactions. The three main methanogenic pathways are hydrogenotrophic, methylotrophic, and acetoclastic. These pathways vary only by substrate use in the initial steps of methanogenesis. Hydrogenotrophic methanogens use H_2, formate, or alcohols to reduce CO_2 (Deppenmeier et al. 1996). Most methanogens are capable of hydrogenotrophic methanogenesis, including the groups Methanobacteriales, Methanomicrobiales, Methanosarcinales, and Methanocellales. Acetoclastic

methanogens split acetate for methane production. Acetoclastic methanogenesis is carried out by methanogens in the Methanosarcina and Methanosaeta groups. Methylotrophic methanogens use methanol or methylamines, which are fermented or reduced by H_2. Methylotrophic methanogenesis can also be carried out by Methanosarcinales (Liu 2010).

It is important to consider peatlands when looking at the effects of climate change because of their large storage of carbon. Peatlands have natural fluxes in temperatures due to seasonal changes, which show increased rates of methanogenesis (Kotsyurbenko et al. 2007). It is predicted that land temperatures will rise 3-5^{o}C globally by the year 2100 (Tarnocai 2006). This is largely an effect of anthropogenic changes to the atmosphere; however other sensitive ecosystems could be affected and contribute to this increase. Peatland soils are expected to have an increase in decomposition as a result of increasing temperatures, which may also lead to drying out of peatlands. Studies have shown that peat emissions of carbon dioxide and methane increased by a factor as high as 6.6 with an increase in temperature (Moore and Dalva 1993). This is likely due to the changes in microbial community composition as warmer temperatures lead to decreased community richness (Kim et al. 2012). Increasing temperatures can also lead to increase in methane production in the case of permafrost thaw in northern peatlands. This thaw allows carbon to decompose quicker and increases temperatures to be optimal for higher microbial activity (Hodgkins et al. 2014).

Changes in water level and soil saturation have been seen to have an effect on peatland microbial activity. While some peatlands may dry out as a result of increasing temperatures and decomposition, some may become saturated due to changes in precipitation, permafrost thaw, and water table levels. Carbon dioxide emissions have been seen to rise when comparing saturated peat samples to those with a lower water table (Estop-Aragonés and Blodau 2012;

Moore and Dalva 1993). Methane production has been predicted to increase in the case of a higher saturation (Freeman et al. 1996; Juottonen et al. 2005). There is potential for methane production to increase by a factor of 30 in the case of periodic drying and rewetting events (Deppe et al. 2010). Changes in water levels have the potential to affect decomposition rates via microbial community changes that could occur from the changing or aerobic and anaerobic zones in peatlands.

The plant communities of peatlands can also have an impact on the methanogen community structure and methane production. There is a feedback cycle between peatland vegetation and biogeochemistry. Peat created from decomposition of different types of plants can have varying hydraulic conductivity and chemicals present (Limpens et al. 2008). *Sphagnum* covered peatlands tend to have a lower decomposition rate (Rydin et al. 2006), whereas sedge or *Carex* dominated peatlands have a higher decomposition rate associated with higher methane emissions (Nilsson et al. 2001; Thomas et al. 1996). The vegetation that covers a peatlands affects the amount of carbon that is being decomposed and how quickly it is being decomposed (Neff and Hooper 2002). Changes in water tables can also have an effect on peatland vegetation as it can lead to interspecific competition and differential growth (Rydin et al. 2006).

Since methanogen communities rely on specific substrates and nutrients to perform their metabolism, the quality of organic matter and the availability of election acceptors influences the microbial pathways of methanogens. Elements and metals in peatland soils can play a role in enzyme activity associated with the decomposition or methanogenesis (Limpens et al. 2008). Among these are heavy metals, such as Ni, Co, Mo, and Fe (Basiliko and Yavitt 2001; Evranos

and Demirel 2015; Hu et al. 2008; Kida et al. 2001). Methanogens are sensitive to the concentration of these metals and other trace metals in the soils as they can stimulate methanogenesis in the correct concentrations and can be toxic when concentrations are too high (Mudhoo and Kumar 2013; Zayed and Winter 2000). Different methanogenic groups have been seen to be more or less tolerant of certain metals and their concentrations. Hydrogenotrophic methanogens have been seen to be less tolerant of high concentrations of Ni and Cu in soils, which can have an effect on their rate of methanogenesis (Kim et al. 1996; Paulo et al. 2017).

Peatlands are important ecosystems to consider in the future of climate change because of their ability to store and release large amounts of carbon. Peatlands vary largely in their environmental makeup, and certain factors seem to have an impact on how sensitive their ecosystems are. The methanogenic archaea that reside in peatland soils and are responsible for their methane production depend on the peat soil for their specific substrates and environmental conditions. Changes to factors such as temperature, pH, nutrient availability, and water level can cause selection for certain methanogenic groups, affecting the diversity and evenness of a community. Disruptions to peatland environments and microbial community structure could lead to increases in the release of carbon in the form of greenhouse gases like methane. It is importation to understand how these methanogenic communities interact with each other and their environment to consider how their carbon cycling could be affected in the process of climate change.

Chapter 2: Methanogen Communities in Peatlands of North America

Introduction

Peatlands are unique acidic wetlands that play a role in the storage and release of atmospheric carbon in the form of carbon dioxide and methane gas (Kennedy and Smith 1995). The accumulation of peat in these environments is a result of relatively low rates of microbial decomposition compared to higher rates of net primary productivity, which leads to peatlands holding an estimated one-third of the Earth's terrestrial carbon (Bradley 2001). Thus, biological processes and characteristics of peatlands are important to investigate for the study of climate change, as changes to these environments could affect their carbon cycling and greenhouse gas emissions.

Microorganisms in peat contribute to carbon cycling, yet these communities are not well understood due to the difficulty of culturing and classifying these organisms. In the anoxic layer of peat, methanogenic Archaea produce methane gas that contributes to climate change. The cultured methanogens span across seven orders (Borrel et al. 2013; Dridi et al. 2012; Lang et al. 2015; Zinder 1993); although genomic analyses indicate there may be eight (Vanwonterghem et al. 2016), or more (Adam et al. 2017) with different specific substrate, nutrient, and environmental requirements (Zinder 1993). There are three main pathways that methanogens use harness energy and create methane as a byproduct. These pathways include methylotrophic, hydrogenotrophic, and acetolactic mechanisms (Ferry 2011). The community composition of methanogens may vary within differing peatland environments; thus, an in-depth analysis of the

methanogenic assemblages across a variety of peatlands was conducted to evaluate links between taxonomic diversity and environmental factors such as pH, temperature, dominant vegetation (e.g. *Sphagnum* coverage) and concentrations of various macro and micronutrients and potential toxicants. In order to better predict changes in methanogenic activities in changing environments, understanding the community makeup across these habitats is crucial.

To analyze the archaeal community, Illumina amplicon sequencing of the *mcrA* gene was performed on peat samples from 17 peatland sites across a latitudinal gradient of North America. The *mcrA* gene codes for the enzyme involved in the last step of methanogenesis, therefore its DNA sequence is used to select for methanogenic Archaea and other *mcrA*- containing organisms involved in reverse methanogenesis or methanotrophy (McKay et al. 2017). A previous study using the 16S rRNA gene has shown that relative abundance of Bacteria and Archaea generally increased with depth in peat soils. In the 16S data, members of the methanogen-containing phylum *Euryarchaeota* did show variance among different peatland classifications; however, perhaps due to low sequence abundance for Archaea, the *Euryarchaeota* did not show any clear trends of community structure based on depth, latitude, or pH in this dataset (Seward et al. 2020). However more specific analysis targeting methanogenic communities could show a trend across sites or other environmental factors. Previous studies indicate that methanogens are typically more active in sites that have higher pH values as indicated by higher methane production, possibly due to increased bacterial activity resulting in bacterial production of methanogenic precursors (Fierer and Jackson 2006; Kennedy and Smith 1995). A previous study showed greater rates of methane production in fens, suggesting both a higher rate of supply of methanogenic substrates and reduced constraints on microbial activity (Lin et al. 2012b). Other environmental factors such as soil metal concentrations can also have

an impact on methanogen community make up and methanogenesis (Chen et al. 2008). While transition metals are needed in the process of methanogenesis, concentrations that are too high have been seen to be toxic to methanogens (Chen et al. 2008; Daas et al. 1994). The study herein uses analysis of *mcrA* genes to provide a more in-depth and focused analysis of the communities responsible for methane production in 17 North American peatlands.

Methods:

Sample Collection

Core samples of peat extracted from 17 peatlands in eastern North America in collaboration with the JGI Global Peatlands Microbiome Project (GPMP) were selected for this study (Table 1). Multiple contributors to the GPMP project provided peat samples as well as environmental data for each peatland site. The peatlands locations used in this study range in latitude from 36.08º to 52.72º. The locations of these peatlands include North Carolina, USA (Pineola, Sugar Mountain, Tater Hill); Tennessee, USA (Ripshin); West Virginia, USA (Cranberry Glades, Big Run); New York, USA (McLean, Purvis Road/Dryden Bog); and Ontario, Canada (Cartier, Daisy Lake, Long Lake, MerBlue, Whitson Lake, Victor Mine). Each peatland was core sampled in triplicate at depths of 10-20cm, 30-40-cm, and 60-70cm beneath the surface. Peat samples were frozen at -20ºC and sent to the USFS lab in Houghton, MI for DNA analysis (Harbison et al. 2016).

Environmental and Chemistry Data

Environmental data was collected for each peatland sample at each site, including conductivity, core temperature, soil pH, *Sphagnum* and vegetation cover, and water-table depth. The average air temperature of the peatland locations was acquired from the national Oceanic

and Atmospheric Administration (NOAA) for sites in the United States, and from the Government of Canada's Environmental and Natural Resources for sites in Canada. Elemental analyses were also performed for each peat sample (Carson 2018). The chemical qualities used in this study include C, Ca, N, Ni, Cu, Mo, W, K, Mg, Co, Na, V, Mn, and Fe concentrations. Prior to elemental analysis on a Varian 810 ICP_MS, peat was ashed and fully acid digested (Watkinson et al. 2017).

Microbial Sequence Analysis

DNA was extracted from peat samples from each of the chosen peatland sites using the QIAGEN DNeasy PowerSoil Isolation kit and then cleaned using the PowerClean kit. The manufacturer's protocol was followed including an added heating step after bead beating (65ºC for 30 minutes) during DNA extraction. PCR was preformed according to the protocol for *mcrA* targeting in soil samples (Juottonen et al. 2006). Initial denaturation at 95ºC for 5 min, 35 cycles of 95º C for 45 s, annealing at 46ºC 45 s, extension at 72ºC for 7 min. The *mcrA* primers mlasF-mod (5'- GGY GGT GTM GGD TTC ACM CAR TA-3') – mcrAR (5'-CGT TCA TBG CGT AGT TVG GRT AGT-3') (Angel et al. 2012; Juottonen et al. 2006; Luton et al. 2002) were used to amplify the *mcrA* gene. PCR products were confirmed by gel electrophoresis in a 1% agarose gel.

Amplified DNA was sent to Metagenombio Inc. for Expression Analysis Illumina Sequencing. Raw sequences were quality filtered and trimmed using the BBMap (Bushnell 2016) package. Forward reads were aligned and processed with QIIME (Caporaso et al. 2010) and USEARCH (Edgar 2010) using an 86.5% confidence threshold for OTU assignments. Taxonomy was assigned using a custom *mrcA* database through DADA2 (Callahan et al. 2016). Bioedit (Hall 2011) was used to create multiple sequence alignments. Phylogenetic programs

Phylip (Felsenstein 1993) and MEGAX (Kumar et al. 2018) were used to view phylogeny and create phylogenetic trees of protein and nucleotide sequences using known sequences from the NCBI database. For comparative purposes, the relative abundance of methanogenic orders were compared to a similar dataset of the 16S rRNA gens in a previously published study (Seward et al. 2020).

Peatland sites were classified into four different categories of peatland based on PI observations, soil pH, and soil calcium concentration. Sites with low pH and calcium were classified as bogs. The sites with either low calcium concentration or low pH were classified as poor fens. The sites with medium range pH and calcium concentrations were classified as intermediate fens. The sites with high range pH and calcium concentrations were classified as rich fens (Figure 2).

Results and Discussion

Methanogen Community Phylogenetics

Phylogenetic analysis of the *mcrA* gene sequences showed that the peatland sites included OTU representatives from five different orders of methanogens that are known to be common in peat: Methanomassiliicoccou s(3 OTUs), Methanomicrobiales (13 OTUs), Methanocellales (5 OTUs), Methanosarcinales (14 OTUs), and Methanobacteriales (2 OTUs). *Methanosarcinales* were the most abundant order across all sites (Supplemental Figure S1A). This supports profiles of mesotrophic and oligotrophic fens in Finland using the *mcrA* gene (Juottonen et al. 2005), and in Canada using the SSU rRNA gene (Godin et al. 2012). When compared across peatland type (Supplemental Figure S2), the abundance of Methanomicrobiales (fen cluster) increased in rich

and intermediate fens, compared to bogs and poor fens; however, the relative proportion of Methanomicrobiales was still quite small (representing less than 30% of sequences). In contrast, SSU rRNA gene (16S) profiling from these same sites demonstrated a predominance of Methanomicrobiales (representing greater than 50% of total sequences) (Supplemental Figure S1B) supporting most other studies (Basiliko et al. 2003; Chroňáková et al. 2019; Galand et al. 2003; Juottonen et al. 2015; Lin et al. 2012a; Martí et al. 2015; Narihiro and Sekiguchi 2011). It is possible that the difference between the SSU rRNA gene and *mcrA* gene data may be due to primer bias inherent in functional gene analyses (Gaby and Buckley 2017) therefore, unweighted analysis methods were used for peatland methanogen community comparison. The phylogenetic trees for both the protein (Figure 3) and the nucleotide (Figure 4) *mcrA* sequences support the presence of peatland methanogenic groups in the 17 sites that were sampled.

Methanogen Community Composition

NMDS and MDS ordination plots were created using Jaccard method for distance. The NMDS plot of *mcrA* methanogen presence and absence (Figure 5) showed a correlation between the pH value (greater than or lower than 5) and specific methanogenic groups. Lower pH values (below 5) were associated with the hydrogenotrophic groups Methanomicrobiales and Methanocellales, as well as the reductive methylotrophic group Methanomassiliicoccous, supporting results from ombrotrophic bogs (Basiliko et al. 2003; Lansdown et al. 1992; Metje and Frenzel 2005; Popp et al. 1999). The peat samples with a pH equal to or greater than 5 were affiliated with the hydrogenotrophic Methanobacteria and the acetoclastic Methanosaeta, supporting results from fens (Godin et al. 2012; Juottonen et al. 2005). This suggests that pH plays a role in shaping methanogen community assemblages in peatlands, corroborating work by Seward et al. 2020. Furthermore, results suggest a shift from hydrogenotrophic to acetoclastic

methanogenesis with increasing pH, supporting work by Conrad et al. 2020. Perhaps consistently, Methanosarcina, which are capable of thriving in a wider range of pH environments (Hunger et al. 2015), did not clearly associate with either the low or high pH sites.

Simpson and Shannon indices were calculated for each peat sample to view the evenness and diversity for each site. The values were averaged and sorted by depth and by peatland classification (Table 2). The average Shannon Index values increased across peatland classifications with increasing nutrient availability and increasing pH, being lowest for the bogs and poor fens, and highest for the intermediate and rich fen sites. The Shannon Index results are consistent with previous studies since low pH has been shown to limit diversity in peatlands and other soils (Lin et al. 2012b; Williams and Crawford 1985). Bogs tend to have fewer, dominant-taxa wile fens tend to have more taxa that are distributed evenly (Galand et al. 2005). Additionally, peatlands that allow acetolactic methanogens to thrive along with hydrogenotrophic methanogens generally have higher diversity among methanogens (Schulz et al. 1997). Thus, the composition of methanogenic communities depends on the quantity and quality of substrates available as well as the environmental conditions (Kotsyurbenko et al. 2019). When looking at diversity by sample depth (Table 2), the Shannon index values were highest at 30cm below the surface, slightly lower at 60cm below, and lowest at 10cm below the surface of the soil. This is consistent with other studies that found the highest diversity and/or evenness of methanogenic populations near 40 cm depth (Cadillo-Quiroz et al. 2006; Galand et al. 2002).

Environmental Influence on Methanogen Community Composition

A NMDS plot was created to visualize which environmental factors had the most impact on the diversity between sites (Figure 6). *Sphagnum* cover, temperature, soil pH, and elemental concentrations were considered for this plot, and compared to the Jaccard distances of the peatland methanogenic assemblages. The NMDS plot showed that nickel concentration had the biggest impact on the community composition. Other factors shown to drive methanogen community structure included temperature, *Sphagnum* cover, and pH of the soil. *Sphagnum* cover likely had an effect on peatland site diversity because the amount of vegetation often correlates with the kinds of methanogens that are present in a site. Acetolactic methanogens tend to thrive in peatlands that have *Carex* sedges and are nutrient rich (Kelly et al. 1992). Peatlands that are dominated by *Sphagnum* typically have an acidic pH and low nutrient concentrations. These *Sphagnum* covered bogs contain microbes that use CO_2 reduction as a methanogenic pathway and are able to thrive in low nutrient environments (Galand et al. 2005; Keller and Bridgham 2007). Soil pH effect on site diversity was also expected because soil pH tends to depend on the nutrients in the soil and the vegetation. Peatlands with lower pH values tend to have less biodiversity and evenness that peatlands with higher pH values. Low soil pH has been seen to be favorable for hydrogenotrophic methanogenesis (Conrad 2002). The temperature effect on peatland diversity could be due to the geographical location itself. Warmer peatlands tend to have higher rates of decomposition (Seward et al. 2020). The community composition of methanogens can be altered by the soil temperature (Kotsyurbenko et al. 2019) and/or regional climate factors . Methanogenic diversity appears to increase in correlating with increasing temperature (Utsumi et al. 2003), however certain methanogenic groups such as *Methanobacteria*, *Methanosarcina* and Methanomicrobiales are abundant in and dominant in colder peatlands (Kwon et al. 2017).

Ni showed a large effect on the methanogen community structure, whereas it had no effect when looking at the general microbial structure (primarily bacteria) of the same samples (Seward 2020). Ni is important in the process of methanogenesis as it is present in Ni-Fe hydrogenases and cofactor F430 (DiMarco et al. 1990). Low bioavailability of Ni limits methanogenesis, however, high concentrations of Ni have potentially detrimental effects on methanogens and methane productions (Chen et al. 2008). Figure 8 shows an NMDS plot of the peatland sites organized by Ni concentration. This plot shows that the peatlands with low concentrations of Ni are have communities more similar to Methanocellales and Methanosarcina. This agrees with literature that states that these methanogenic groups are inhibited by higher concentrations of Ni in soil (Paulo et al. 2017; Wang et al. 2019). The Methanomicrobiales in this groups seem to be able to thrive in peatlands with middle to high concentrations of Ni. This could be due to the diversity of Methanomicrobiales or due to other factors in the environment that are affected by the high metal concentrations, such as lowered pH and *Sphagnum* loss (Carson 2018).

Due to the impact of Ni, other metal concentrations in the peatland soils were observed for community structure impact through an NMDS plot (Figure 7). Results demonstrated that Cu can also drive methanogen assemblages in peatlands. Needed only in trace amounts, Cu is an important transition metal in methane production, and is even more vital in the process of methanotrophy (Glass and Orphan 2012). In high concentrations, Cu is known to be toxic to acetolactic and hydrogenotrophic methanogens (Karri et al. 2006). Other trace metals that drive community structure are Cr, Mo, and W. Mo/W can bind with Fe to help transfer electrons from H_2 to other enzymes of methanogenesis (Daas et al. 1994), and are needed in relatively high

concentrations (Glass and Orphan 2011). Cr is known to have high to moderate importance on microbial metabolic function and can be toxic in high concentrations, although less toxic than Cu (Daas et al. 1994).

Some of the peatland sites in this study (Long Lake, Daisy Lake, and Whitson) are impacted sites from the Copper Cliff smelter in Sudbury, Ontario. These sites have enriched Cu and Ni concentrations in the peat, which was found to negatively impact the *Sphagnum* coverage of these peatlands. Another study found that peatlands close to these Ni and Cu smelters had negatively impacted methanogen abundances and shifted methanogen order prevalence (Carson 2018).

Conclusions

The results showed that when looking at peatlands across eastern North America, trends in pH and environmental factors can be seen to affect the methanogen community makeup of the peatland soils. The phylogenetic results showed that the representative OTU sequences extracted from the peatlands sites formed 5 distinct phylogenetic grouping of methanogens that are known to be present in peatland soils. The values of the Shannon Index show increased evenness and diversity in rich fen sites compared to bogs, poor fens, and rich fens. An increase in community evenness and diversity was also seen with depth, with the highest diversity around 30cm depth samples, where the methanogenic zone is likely to be.

The impact of pH can on the community structure of the peatland sites can be seen when looking at the trend in peatland sites shifting from hydrogenotrophic groupings to acetolactic with increasing pH. The peatland sites with a pH below 5 tend to have communities with more similarity to methanogen groups Methanomassiliicoccous, Methanomicrobiales,

Methansarcinales, and Methanocellales. The peatland sites with a pH of 5 or greater had communities more similar to methanogen groups Methanobacteria, Methanosaeta, Methanosarcina, and Methanocellales. The impact of pH on community structure can also been seen when looking at the environmental factors with site diversity.

Other environmental factors are also seen to have an impact on methanogen community makeup in the peatland sites. *Sphagnum* coverage, annual temperature, as well as Ni concentration are shown as drivers of community diversity. The varying environments of these different peatlands classifications seems to have an impact on how even and diverse the methanogen communities are.

The results also showed that metal concentrations in the peatland soils have an impact on community structure. Ni concentration is shown as the biggest driving factor of site diversity, with other metals such as Cu, Mo, and W also having an effect. The impact of metals on methanogen diversity in these peatlands indicates how important metal concentrations in soils are to methanogens and methanogenesis. Some metals such as Ni can help in the process of methanogenesis while some metals such as Cu can be detrimental if their concentrations are too high.

The results of this study showed that there are many environmental factors that have an effect on peatland community structure. This shows that changes to the environment as a result of climate change could have an impact on methanogen communities by affecting which methanogenic groups are dominant as well as the community evenness in a peatland. Climate change has the potential to affect the temperature and the water levels of the earth which can result in shifts in vegetation and pH in peatland environments. The results of this study showed that some of the main driving factors of diversity in the peatlands were pH, temperature, and

Sphagnum coverage. Since peatlands are such large carbon sinks, disturbances to their community structure has potential to cause the release of stored carbon in the form of methane and carbon dioxide. It is important to consider how environmental shifts in these peatland environments could cause changes to the methanogenic communities in the soil and result in changes to the carbon cycling and greenhouse gas production.

Tables

Table 1. Peatland sites selected for this study along with the location, pH, elevation, coordinates, and average air temperature for each site.

Peatland	Location	Peatland Classification	Average pH	Elevation (m)	Latitude (decimal degrees)	Longitude (decimal degrees)	Average Annual Temperature (°C)	Depth to Water Table (cm)
Victor Mine VICM	Hudson Bay Lowlands, Ontario, Canada	Poor Fen	4.01	88	52.7208123	-83.940048	-0.56	5
Cartier	Sudbury gradient, Ontario, Canada	Bog	4.02	423	46.662818	-81.520331	4	25
MerBlue	Ontario, Canada	Bog	4.02	69	45.41	-75.48	4	40-46
Victor Mine VMOE Bog	Hudson Bay Lowlands, Ontario, Canada	Bog	4.04	91	52.5051329	-83.802497	-0.56	36-48
Cranberry Glades	West Virginia, USA	Bog	4.06	1026	38.2008	-80.272	10.16	15-20
McLean	New York, USA	Bog	4.09	341	42.548812	-76.266274	8.14	5
Purvis Rd/Dryden Bog	New York, USA	Poor Fen	4.31	372	42.447156	-76.258488	8.14	10
Daisy Lake	Sudbury gradient, Ontario, Canada	Poor Fen	4.61	249	46.45491	-80.88248	3.5	7-15
Big Run	West Virginia, USA	Poor Fen	4.67	981	39.116859	-79.581104	9.69	5
Long Lake	Sudbury gradient, Ontario, Canada	Poor Fen	5.04	286	46.221583	-81.037083	3.5	30-53
Sugar	North Carolina, USA	Intermediate Fen	5.13	1230	36.0838502	-81.893484	10.14	8-22
Whitson	Sudbury gradient, Ontario, Canada	Intermediate Fen	5.45	299	46.590095	-80.991547	3.5	14-22
Pineola	North Carolina, USA	Intermediate Fen	5.46	1066	36.023497	-81.89836	10.14	17-38
Ripshin	Tennessee, USA	Intermediate Fen	5.56	1085	36.1659	-82.1529	13.4	17-35
Tater Hill	North Carolina, USA	Rich Fen	5.96	1258	36.283747	-81.715354	10.14	12-14
Victor Mine VMOE Fen	Hudson Bay Lowlands Ontario	Rich Fen	6.52	88	52.698933	-83.953692	-0.56	20
Cedar	Ohio, USA	Rich Fen	7.79	295	40.058813	-83.794255	10.5	12

Table 2. Average Shannon Index values and Simpson Index values for peatland sites organized by each peatland classification and depth below soil surface.

	Shannon Index	Simpson Index
Rich Fen	0.8325	0.5623
Intermediate Fen	0.7555	0.5771
Poor Fen	0.7094	0.5074
Bog	0.5480	0.6920
10cm	0.2491	0.8633
30cm	0.4145	0.7473
60cm	0.3895	0.7341

Figures

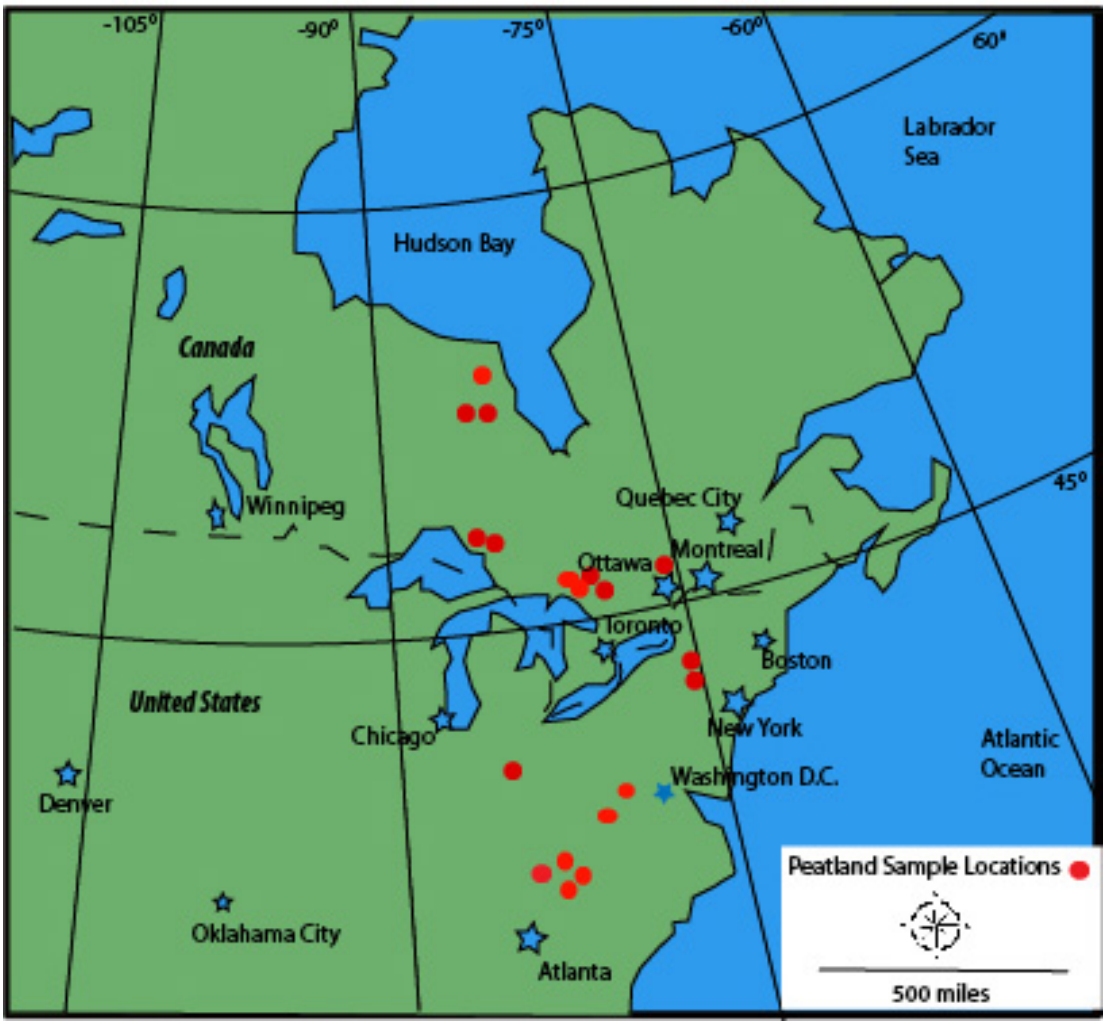

Figure 1. Map of peatland locations used in this study.

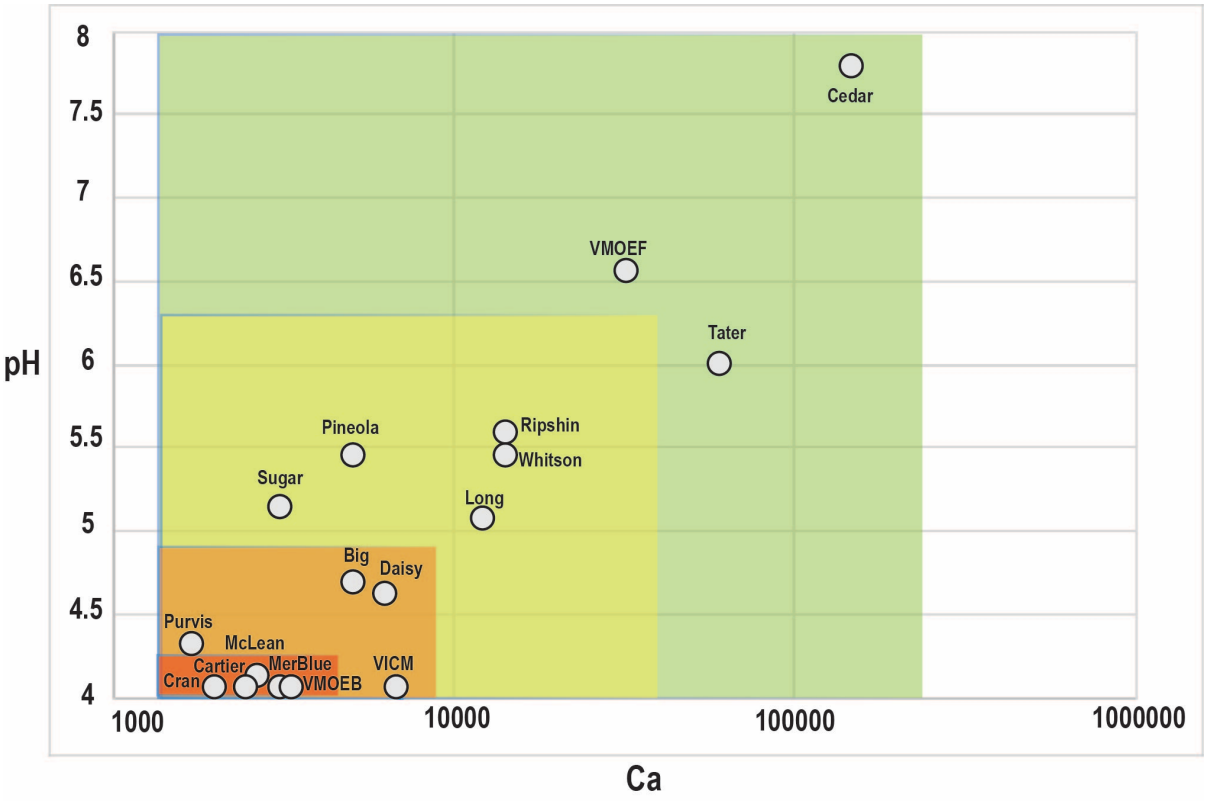

Figure 2. Peatland sites classified by Ca concentration and pH. Red = bog, orange = poor fen, yellow = intermediate fen, green = rich fen.

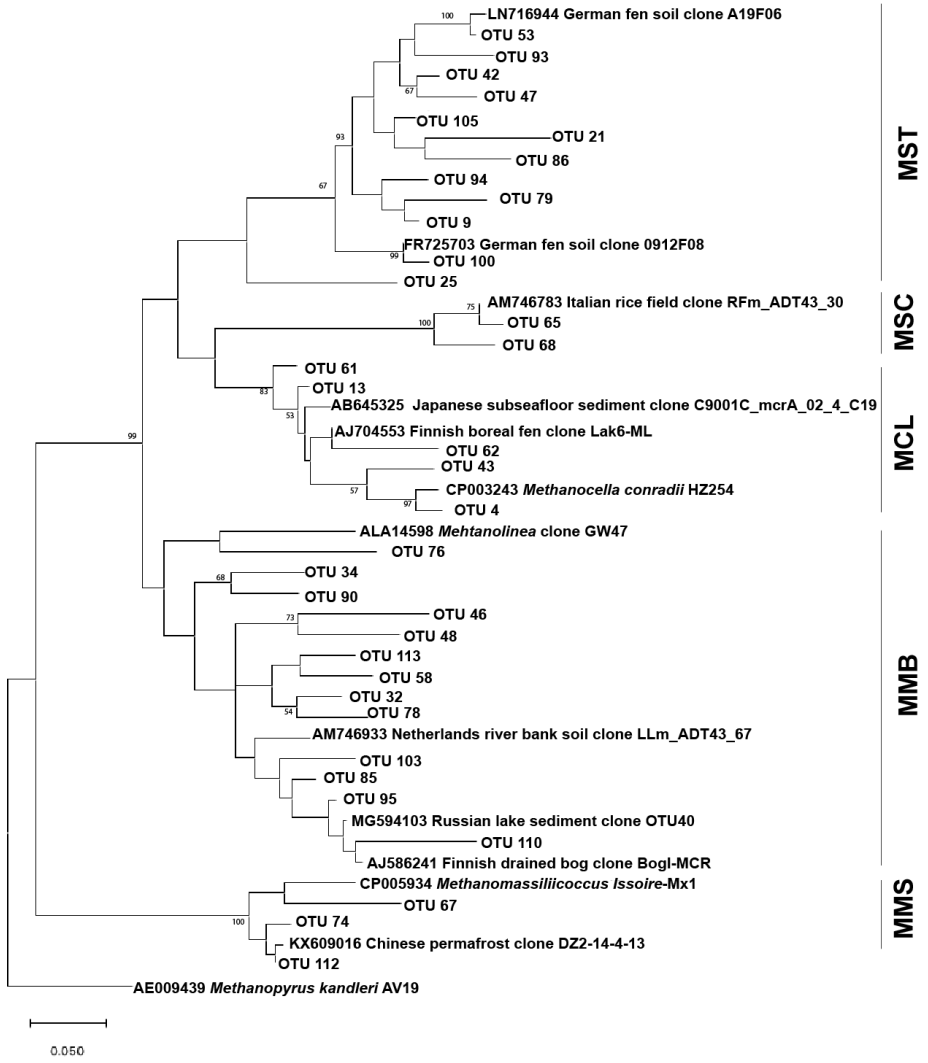

Figure 3. Neighbor-Joining phylogenetic tree of protein *mcrA* sequences.

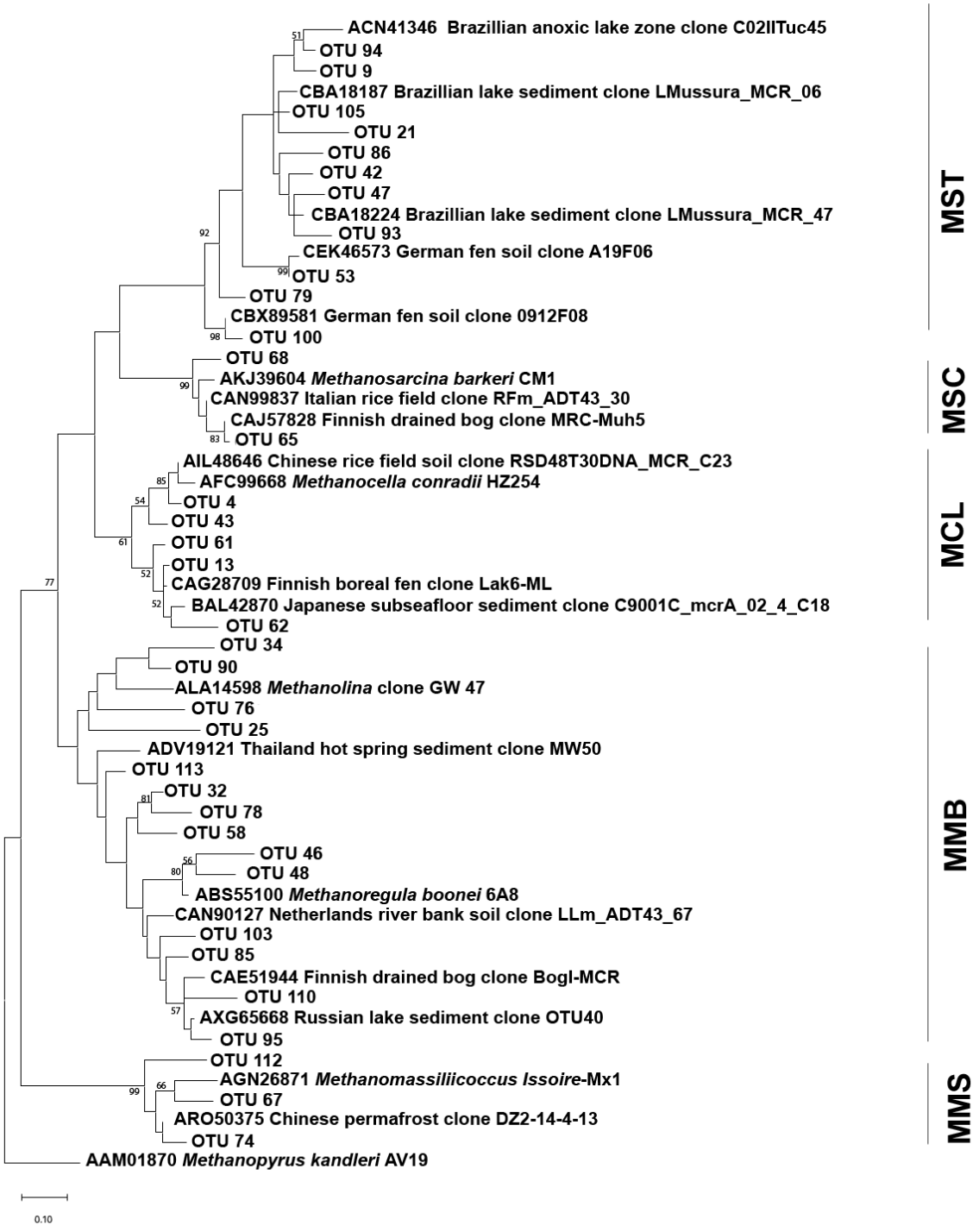

Figure 4. Neighbor-Joining phylogenetic tree of nucleotide *mcrA* sequences.

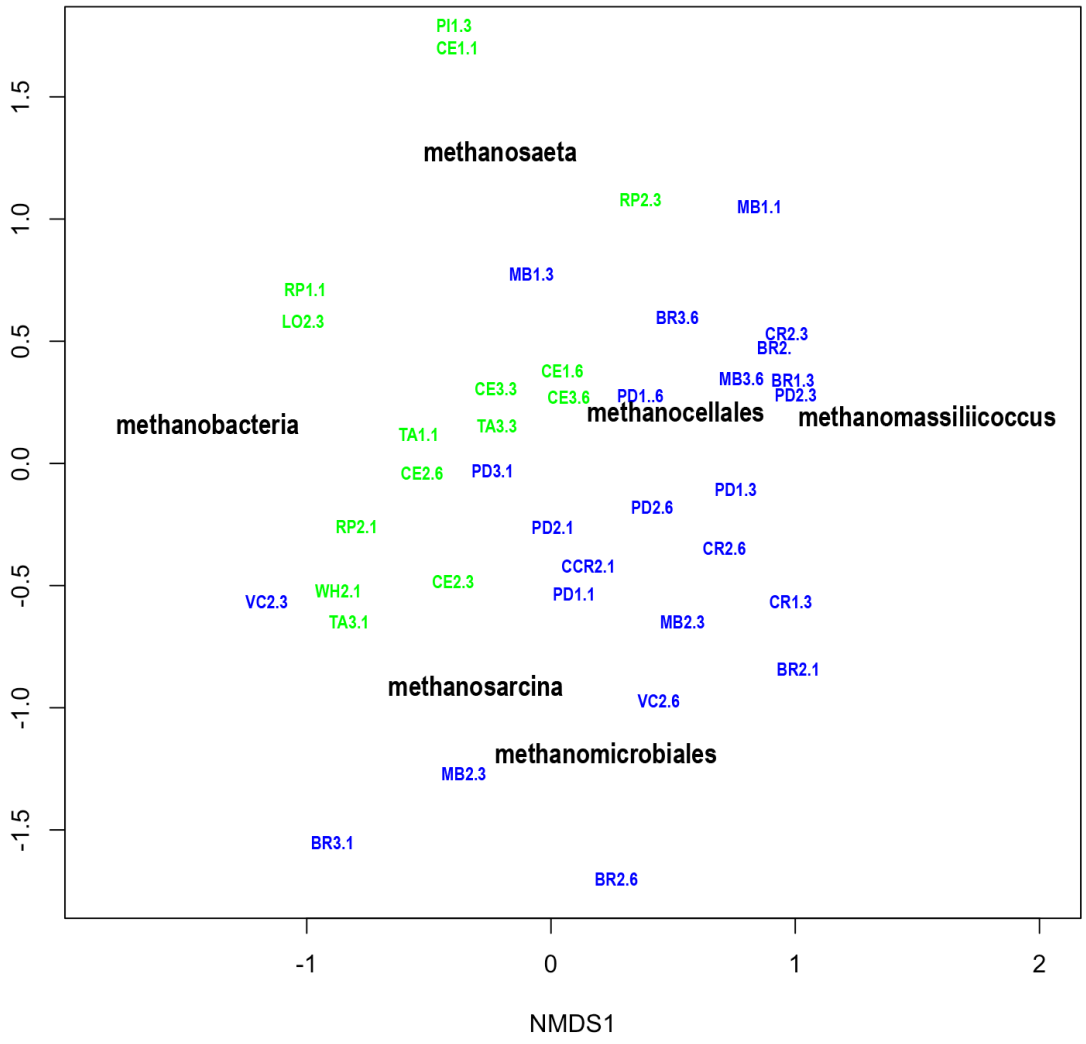

Figure 5. NMDS plot with Jaccard distance of peatland sites with methanogenic groups showing the effect of pH on site diversity. Sites colored blue have a pH below 5. Sites colored green have a pH of 5 or higher.

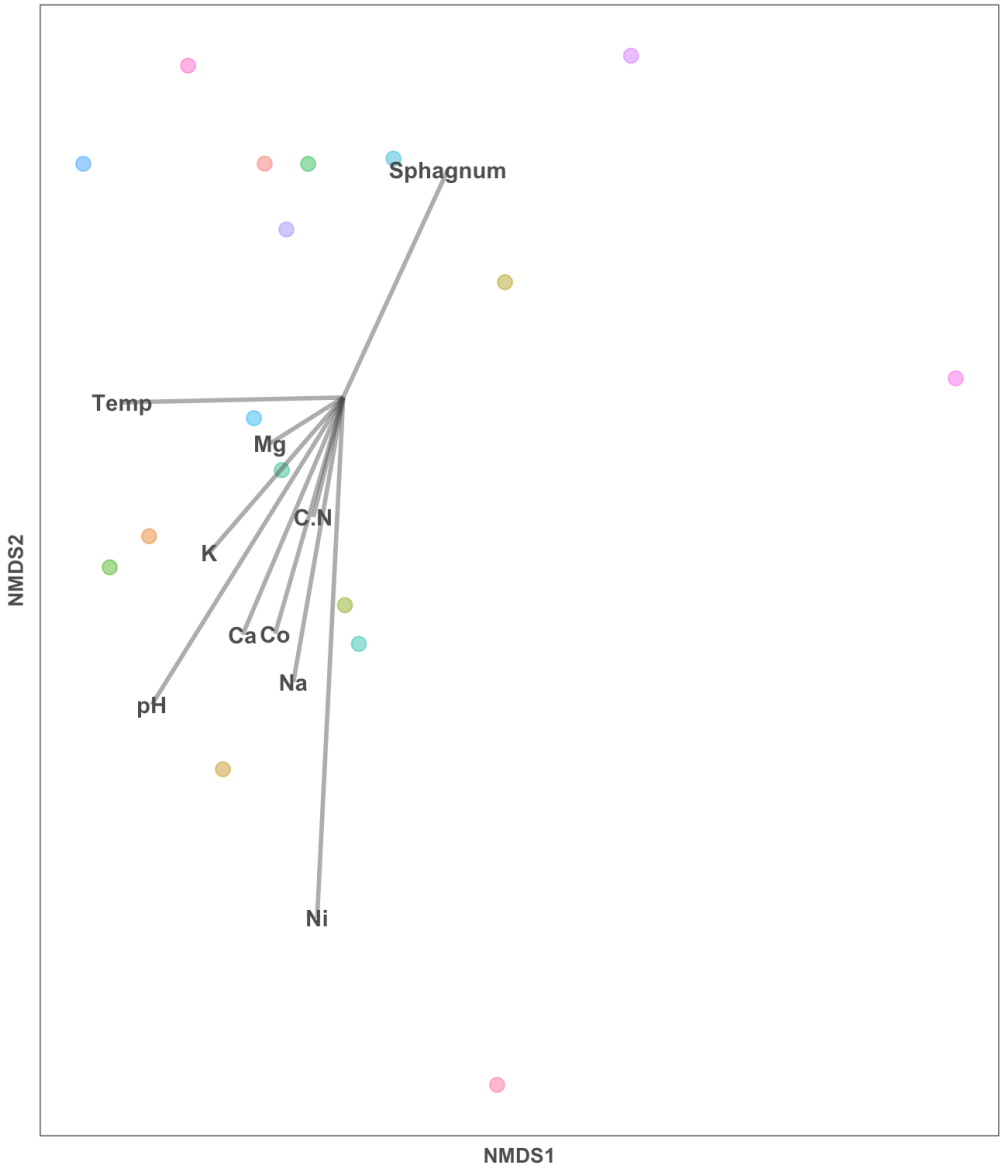

Figure 6. NMDS plot with Jaccard distance of peatland sites overlaid with environmental factors.

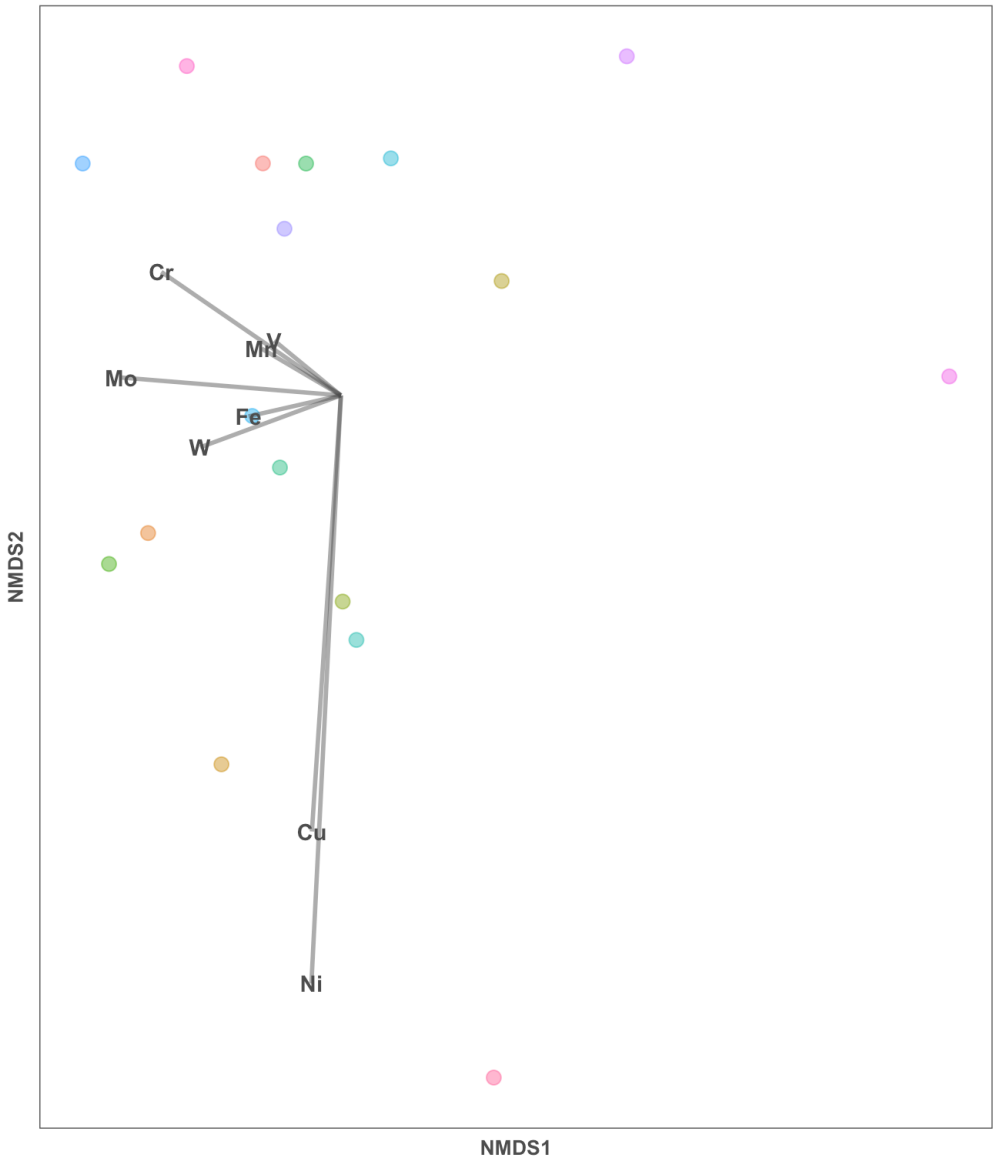

Figure 7. NMDS plot with Jaccard distance of peatland sites overlaid with soil metal concentrations effect on sites.

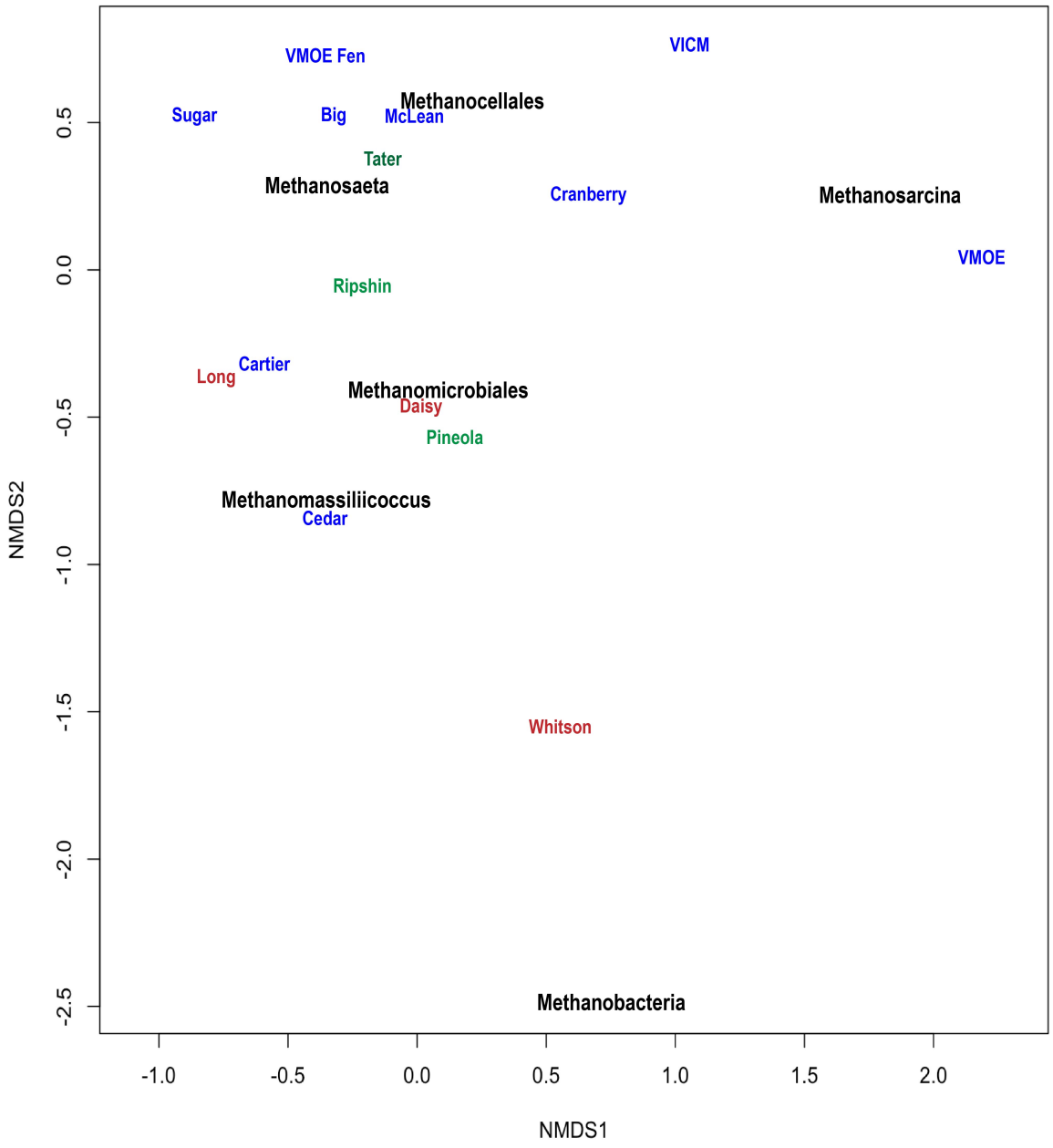

Figure 8. NMDS plot with Jaccard distance of peatland sites with methanogenic groups showing the effect of soil Ni concentration on site diversity. Sites colored blue have a Ni concentration <50mg/kg, sites colored green have a Ni concentration 100-300mg/kg, and sites colored red have a Ni concentration >500mg/kg.

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Appendix A: Supplemental Figures

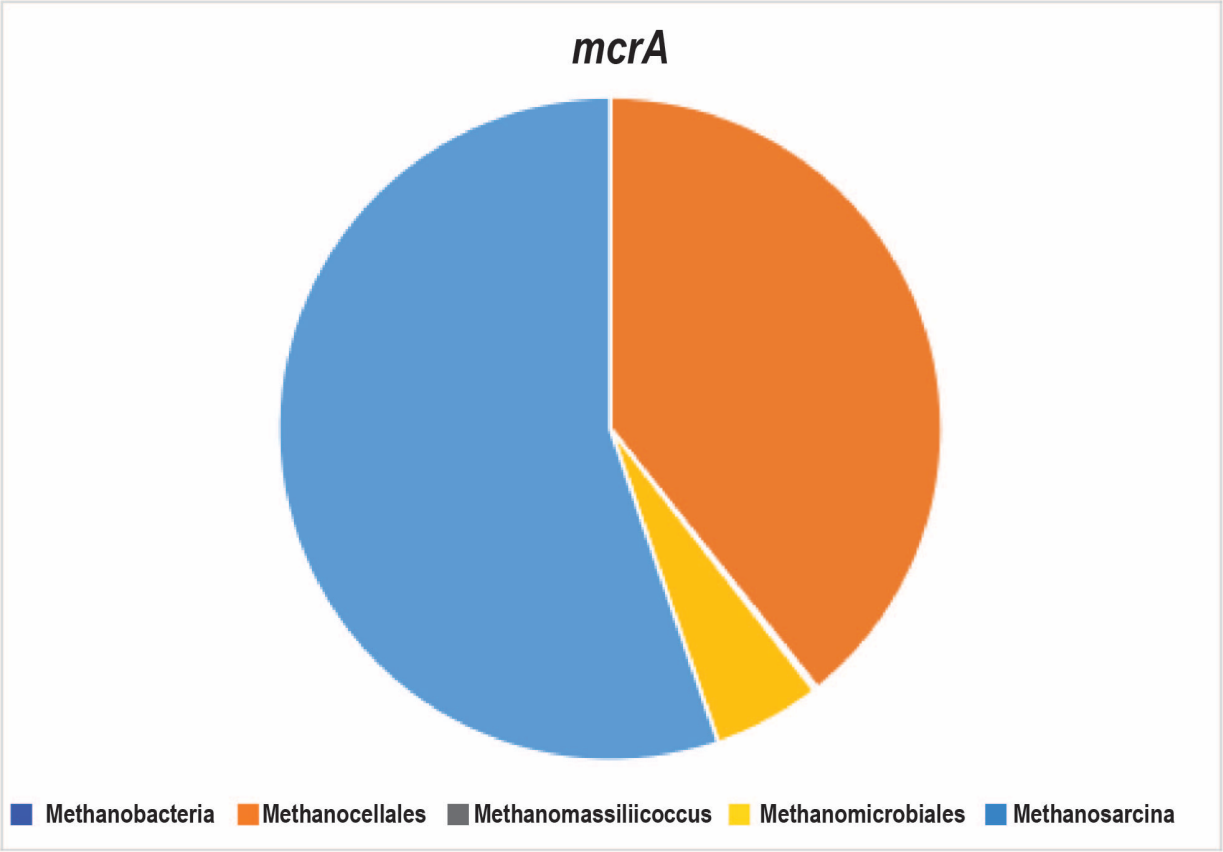

Figure S1A Total copy number of mcrA sequences by methanogenic order

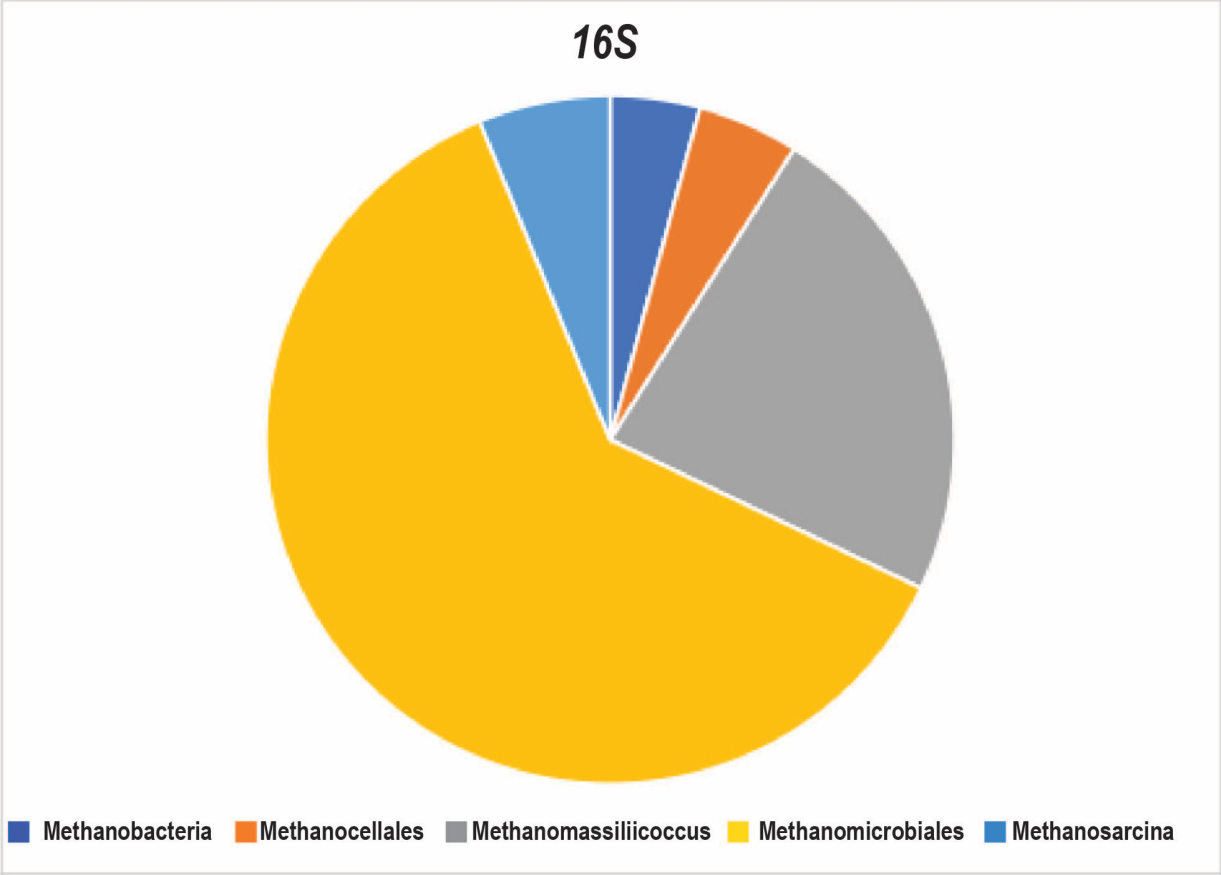

Figure SIB Total copy number of 16S sequences by methanogenic order

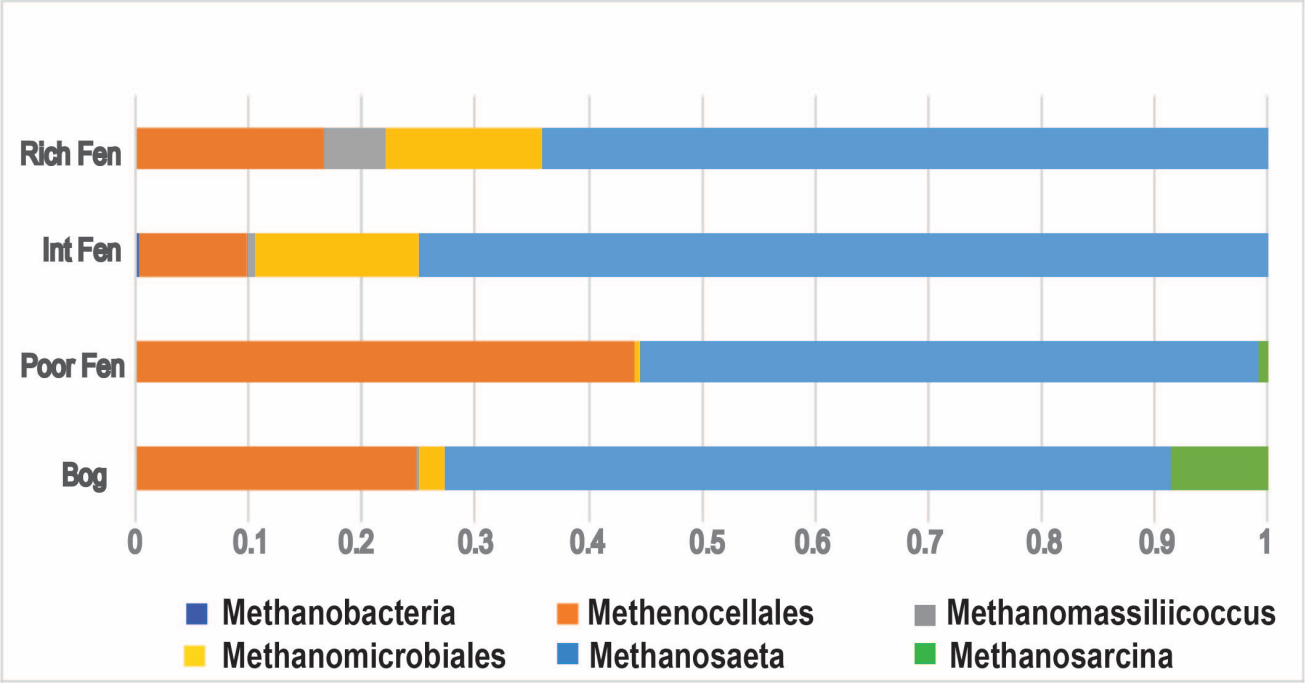

Figure S2 Average methanogen community composition of peatland sites organized by peatland classification

Vita

Sydney Elizabeth Bear was born in Raleigh, North Carolina on the 7th of June 1996. Sydney graduated from Appalachian State University with a B.S. in Cell and Molecular Biology in 2018. During her undergraduate studies she realized her interest in microbiology and began research in Dr. Suzanna Bräuer's lab. She continued her education at Appalachian State University and completed her M.S. in Cell in Molecular Biology in August 2020.